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(54) Title: METHOD AND DEVICE FOR DELIVERY OF APOPTOSIS-INDUCING MOLECULES

(57) Abstract

A device and method for capsular delivery of an apoptosis-inducing molecule to a patient, preferably to the central nervous system.

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METHOD AND DEVICE FOR DELIVERY OF APOPTOSIS-INDUCING MOLECULES

TECHNICAL FIELD OF THE INVENTION

This invention relates to a method and device for delivery of Fas ligand using encapsulated cells.

BACKGROUND OF THE INVENTION

Apoptosis is a distinct form of cell death which is essential for the regulation of cellular homeostasis. In the immune system, Fas/APO-1 (CD95) and its ligand (FasL) appear to play an important role in various processes involving the induction of apoptosis. So far the FasL-Fas/APO-1 system has been implicated in T cell mediated cytotoxicity, in the regulation of T cell homeostasis and possibly in thymocyte selection and development. It has been shown that the FasL can exert its cytotoxic activity as a membrane-bound molecule, requiring cell-to-cell contact with the Fas/APO-1 expressing target cells. However, significant cytotoxic activity has also been found in the supernatant of COS cells transfected with the rat FasL cDNA. Moreover, supernatant of cultured activated T cells and T cell hybridomas has recently been shown to contain FasL which can induce T cell apoptosis in an autocrine or paracrine manner *in vitro*.

The impressive efficacy of the death pathway mediated by cross-linking of the Fas/APO-1 receptor *in vivo* has been demonstrated by the administration of antibodies directed against human Fas/APO-1. A single injection of anti-Fas/APO-1 antibodies into nu/nu mice carrying a xenotransplant of a human

B cell tumor induced tumor regression within a few days. Unfortunately, the establishment of a syngeneic model for Fas/APO-1-directed tumor therapy in mice has been hampered by the finding that antibodies to murine Fas/APO-1 induce lethal liver damage within 3 to 6 h after intraperitoneal injection. Thus, if soluble FasL was indeed involved in mediating T cell apoptosis *in vivo*, control mechanisms may exist which ensure specificity and prevent systemic toxicity.

The Fas receptor is typically expressed in mitotic tissues, but not in post-mitotic tissues, such as the mammalian central nervous system ("CNS"). In particular, in neural tissue, astrocytes, oligodendrocytes, and neurons do not express APO-1 (the Fas receptor) and are resistant to FasL mediated apoptosis. However, many tumor cells, including glioblastomas, express high levels of Fas receptor. In addition, antigen-activated T cells and macrophages express high levels of Fas receptor.

The Fas receptor protein is also expressed in the thymus, liver, heart and ovary. The Fas receptor is up-regulated by IFN-γ and combinations of IFN-γ and TNF-α in human B cells. Any lymphocyte transformed with human T cell leukemia, human immunodeficiency or Epstein Barr virus also expresses high levels of Fas receptor.

In the present invention, devices and methods are provided for delivery of FasL, or other suitable apoptosis-inducing molecule, to a suitable implant site in a patient, preferably directly to the CNS.

SUMMARY OF THE INVENTION

This invention provides novel methods and devices for delivery of a source of FasL, or other suitable apoptosis-inducing molecule. In one embodiment, one or more devices, each containing between about 10^3 - 10^7 genetically modified cells surrounded by a permeable membrane, is implanted into a patient. The encapsulated cells provide for release of FasL or other suitable apoptosis inducing molecule. The device or devices should produce a level of FasL (or other suitable

apoptosis-inducing molecule) sufficient to induce apoptosis of the target cells or tissue.

In a preferred embodiment, one or more devices are implanted into the CNS. Delivery to a localized area in the CNS is achieved, e.g., by implanting encapsulated cells into the brain parenchyma. Delivery over a more widespread area of the CNS is accomplished by implanting encapsulated cells into the brain ventricles or cerebrospinal fluid ("CSF") space.

The device contains a permeable membrane that prevents immunologic rejection of the encapsulated cells and interposes a physical barrier between the cells and the patient. Moreover, each device may be retrieved from the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Supernatant of Neuro-2a FasL transfectants contains Fas/APO-1 specific cytotoxic activity.

(a) Amplification of the full-length FasL transgene by RT-PCR yields a band of 875 bp in Neuro-2a cells transfected with the murine FasL gene (lane 2) but not in the Neuro-2a mock transfectants (lane 1). As a control, PCR with the same primers was carried out using the BCMGS neo vector containing the FasL cDNA (lane 4) or the BCMGS neo plasmid without insert (lane 3) as a template. Size markers are indicated in base pairs (bp) on the left.

(b) Yac-1 target cells were exposed to serial dilutions of concentrated supernatant derived from Neuro-2a FasL (closed circles) and Neuro-2a mock transfectants (open circles) for 24 h. Viability was calculated as percent (^{3}H) thymidine uptake of untreated cells. Addition of soluble FasL ("sFas") sFas (500 ng/ml) (closed triangles) but not sTNF-R (open triangles) completely inhibited the cytotoxic effect of the FasL supernatant on Yac-1 cells.

(c) Yac-1 cells were incubated with increasing concentrations of the anti-Fas antibody Jo2 (closed squares), Jo2 plus protein A (10 µg/ml) (closed triangles) or control hamster IgG (open squares) and viability was determined as described above.

(d) Neuro-2a cells transfected with the cDNA encoding human Fas/APO-1 were stimulated with FasL supernatant (10 U/ml) alone or in the presence of either anti-APO-1 $F(ab')_2$ or FII23 control $F(ab')_2$ fragments (100 ng/ml). All cultures contained cycloheximide (10 µg/ml) and survival was quantified after 16 h by crystal violet staining and calculated as percent survival of cells exposed to cycloheximide alone.

Figure 2: Detection of FasL in the supernatant of Neuro-2a FasL transfectants. Concentrated supernatants obtained from Neuro-2a FasL transfectants (lane 1) or Neuro-2a mock transfectants (lane 2) were subjected to SDS-PAGE and subsequent Western blotting using anti-Fas/APO-1 ligand antibodies.

Figure 3: The FasL induces apoptosis of Yac-1 cells *in vivo*.

(a) Two devices each containing 4-6 x 10^5 Yac-1 cells were implanted into the peritoneal cavity of each mouse. 16 h after intraperitoneal injection of increasing doses of FasL or of control supernatant (ctrl) the mice were sacrificed and Yac-1 cells isolated from each device individually. Viability was assessed by FDA staining; the results are expressed as mean values of two individually analyzed devices.

(b) Yac-1 cells isolated from mice which received FasL containing supernatant (1000 U) i.p. were stained with acridine orange and analyzed by fluorescence microscopy (x 354). The cells exhibit marked nuclear condensation and fragmentation.

(c) Yac-1 cells recovered from mice injected with control supernatant show intact, evenly stained nuclei (x 354).

(d and e) Yac-1 cells which had been exposed to FasL (1000 U) or control supernatant for 16 h *in vivo* were fixed for 10 min in 4% formaldehyde in PBS and processed for *in situ* DNA end labeling. The alkaline phosphatase substrate produces a dark blue stain which allows the identification of DNA breaks on a single cell level (x 708).

Figure 4: Systemic but not local application of FasL-containing supernatant induced liver apoptosis. Groups of weight-matched mice were injected with:

(A) 2000 U of FasL supernatant i.p.,

(B) 500 U of FasL i.v.,

(C) concentrated control supernatant i.v., or

(D) 100 µg/ml Jo2 antibody i.p.

Liver sections were prepared 16, 2, 2 and 4 h after injection, respectively. Sections were stained with haematoxylin and eosin, magnification is 298 x in all cases. Normal liver tissue is seen in (a) and (c), whereas the hepatocytes in (b) and (d) carry pyknotic and fragmented nuclei and are surrounded by red blood cells.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to delivery of FasL or other suitable apoptosis-inducing molecule using encapsulated cells. In one embodiment, localized systemic delivery of FasL is contemplated. This embodiment may provide a therapy for human tumors or cancers. One benefit is the reduction or elimination of side effects resulting from systemic injections or other conventional administration routes. Such side effects include hepatoxicity.

We also contemplate delivery directly into the CNS without having to cross the blood brain barrier. The methods and devices of this invention allow delivery of significant levels of FasL to the CNS. This embodiment may provide a potential therapy for tumors or cancers inside the CNS. In a preferred embodiment, this invention contemplates capsular delivery of an apoptosis-inducing molecule to

the CNS, in a sufficient dosage to result in apoptosis of the target tumor or cancer, but not of the surrounding non-tumorigenic or non-cancerous tissue. In another embodiment, the dosage of FasL provided is sufficient to achieve destruction of activated T cells or macrophages.

Local application of FasL may be useful in the treatment of primary brain tumors, e.g., malignant glioma, and lymphoma of the CNS, as well as metastatic tumors provided they are Fas/APO-1 positive.

Similarly, delivery of FasL into the cerebrospinal fluid (CSF) may be useful in the treatment of meningeal infiltrations with leukemic or carcinoma cells, ependymoma and multifocal gliomas.

In a particular embodiment, delivery of FasL is contemplated for the treatment of glioblastoma. Glioma cells express Fas/APO-1 and are susceptible to anti-Fas/APO-1-mediated apoptosis *in vitro*.

Importantly, Fas/APO-1 expression is not detectable in normal brain tissue including neurons and glial cells, with the exception of endothelial cells. However, it has recently been reported that despite their Fas/APO-1 expression, cultured endothelial cells are not susceptible to Fas/APO-1 mediated cytotoxicity. Moreover, in vitro cultured neurons and oligodendrocytes treated with IFN-gamma and TNF-α resist FasL mediated killing.

Considering the potency of the FasL in eliminating tumor cells without damaging other organs when applied locally, intrathecal or intratumoral application of FasL may be a promising approach for the treatment of Fas/APO-1 expressing malignant tumor cells.

The devices and methods of this invention may also be used as part of a therapy regime that includes other anti-cancer or anti-tumor treatments, such as administration of chemotherapeutic agent(s). The combined treatment of an apoptosis-inducing molecule with a chemotherapeutic agent(s) may provide an enhanced "killing" effect on the target tumor or cancer tissue.

This invention also contemplates the use of encapsulated cells that produce an apoptosis-inducing molecule (e.g. Fas-L) for the treatment of multiple sclerosis (MS), and other forms of immune-mediated demyelinating diseases.

In one embodiment, using a FAS-ligand expressing cell line, we can apply soluble FAS-ligand delivery to the CSF space for the treatment of autoimmune dysfunction (for example, multiple sclerosis). In this CNS autoimmune disease, cytotoxic lymphocytes including T-cells, neutrophils, macrophages, and B-cells are activated and attack the myelin sheaths of CNS axons. Activated macrophages and lymphocytes express high levels of FAS/APO-1 receptor and are susceptible to FAS-ligand mediated apoptotic killing. As activated immune cells traffic into the CNS to attack myelin sheaths, they will be induced to undergo apoptotic death in response to FAS-ligand expression. In that CNS neurons, astrocytes and oligodendrocytes do not normally express the APO-1/FAS receptor, only the activated immune cells will be affected. This may be analogous to the environment created in the eye and testis where high levels of FAS-ligand are expressed and serve to immunosuppress these environments (Griffith et al., Science, 270, pp. 1189-1192 (1995); Suda et al., Cell, 75, pp. 1169-1178 (1993)).

FAS and its receptor are members of the TNF gene family which includes TNF NGFR (low affinity), CD40, OX40, 4-13B, CD27 and CD30 (Suda et al., Cell, 75, pp. 1169-1178 (1993); Rabizadeh and Bredesen, Dev. Neurosci., 16, pp. 207-211 (1994)). TNF and FAS share similar death domains. Interestingly, low affinity NGF receptor may be directly involved in mediating apoptotic actions but in the reverse fashion when the LNGF receptor is not occupied (Rabizadeh and Bredesen, Dev. Neurosci., 16, pp. 207-211 (1994)). LNGF receptor may act directly to prevent apoptosis when occupied with NGF (Ibid.); Dobrowsky et al., Science, 265, pp. 1596-1599 (1994)) or in conjunction with other Trk receptors and BDNF, NT-3 or NT4/5 (Hantzopoulos et al., Neuron, 13, pp. 187-201 (1994)).

For CNS delivery, the contemplated technique provides several advantages over other delivery routes:

(1) Drug can be delivered to the CNS directly, which will potentially reduce unwanted peripheral side effects including the toxicity expected for systemic delivery in human patients;

(2) Very small doses of drug (nanogram or low microgram quantities rather than milligrams) can be delivered compared with bolus injections, also potentially leading to fewer side effects;

(3) In embodiments where the encapsulated cells continuously produce newly synthesized product, these cells should have advantages over pump delivery of drug stores, where drug is continuously degraded (or its potency drops) but is not continuously replenished.

Delivery of FasL through cell therapy (i.e., via transplantation of encapsulated cells that produce or that have been genetically-engineered to release FasL) circumvents the majority of these problems.

The physical and chemical characteristics of FasL are known. FasL is approximately 40,000 molecular weight glycoprotein and member of the tumor necrosis factor family of proteins. The murine gene is composed of five (5) exons and four (4) introns, with an open reading frame coding for 279 amino acids.

The amino acid sequence of FasL contains a region of 22 hydrophobic amino acids in the middle of the molecule, suggesting that FasL is a Type II membrane protein. The protein does not have a hydrophobic signal sequence.

The genes encoding murine and human FasL are known. The preferred FasL is the naturally-occurring murine or human ligand, such as described in EP 675 200, WO 95/32627, Cheng et al., Science, 263, pp. 1759-62 (1994), Suda et al., Cell, 75, pp. 1169-78 (1993) and Takahashi et al., Int. Immunology, 6, pp. 1567 (1994), each of which is incorporated herein by reference.

The entire membrane form of FasL, as well as modified, truncated and mutein forms of FasL, as well as active fragments of FasL (i.e., those fragments of FasL having biological activity) are also known. All of these forms of FasL are

contemplated by this invention. Each of these documents are specifically incorporated herein by reference.

While FasL is the preferred ligand, any other suitable apoptosis-inducing molecule may also be delivered using the methods and devices of this invention. For example, lymphotoxin, CD40L and TNF-α may also useful in the methods and devices of this invention.

A gene of interest (i.e., a gene that encodes a suitable biologically active molecule, e.g., FasL) can be inserted into a cloning site of a suitable expression vector by using standard techniques. It will be appreciated that more than one gene may be inserted into a suitable expression vector. These techniques are well known to those skilled in the art.

The expression vector containing the gene of interest may then be used to transfect the desired cell line. Standard transfection techniques such as calcium phosphate co-precipitation, DEAE-dextran transfection or electroporation may be utilized. Commercially available mammalian transfection kits may be purchased from e.g., Stratagene.

A wide variety of host/expression vector combinations may be used to express the gene encoding FasL, or other biologically-active molecule of interest.

Suitable promoters include, for example, the early and late promoters of SV40 or adenovirus and other known non-retroviral promoters capable of controlling gene expression.

Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences, such as various known derivatives of SV40 and known bacterial plasmids, e.g., pUC, pBlueScript™ plasmids from E. coli including pBR322, pCR1, pMB9, pUC, pBlueScript™ and their derivatives.

Expression vectors containing the geneticin (G418) or hygromycin drug selection genes (Southern, P.J., In Vitro, 18, p. 315 (1981), Southern, P.J. and Berg, P., J. Mol. Appl. Genet., 1, p. 327 (1982)) are also useful. Expression vectors containing the zeocin drug selection gene are also contemplated.

Examples of expression vectors that can be employed are the commercially available pRC/CMV, pRC/RSV, and pCDNA1NEO (InVitrogen). The viral promoter regions directing the transcription of the drug selection and biologic genes of interest are replaced with one of the above promoter sequences that are not subject to the down regulation experienced by viral promoters within the CNS. For example, the GFAP promoter would be employed for the transfection of astrocytes and astrocyte cell lines, the TH promoter would be used in PC12 cells, or the MBP promoter would be used in oligodendrocytes.

In one embodiment, the pNUT expression vector is used. Baetge et al., PNAS, 83, pp. 5454-58 (1986). In addition, the pNUT expression vector can be modified such that the DHFR coding sequence is replaced by the coding sequence for G418 or hygromycin drug resistance. The SV40 promoter within the pNUT expression vector can also be replaced with any suitable constitutively expressed mammalian promoter, such as those discussed above. In this embodiment, the human FasL gene is expressed from the pNUT vector, which contains the cDNA of the mutant DHFR and the entire pUC18 sequence including the polylinker (Baetge et al., supra). The DHFR transcription unit is driven by the SV40 promoter and fused at its 3' end with the hepatitis B virus gene polyadenylation signal (approximately 200 bp 3' untranslated region) to ensure efficient polyadenylation and maturation signals.

The FasL gene is expressed behind the mouse metallothionein I promoter and utilizes the human growth hormone polyadenylation signal sequence for 3' processing.

This invention also contemplates use of a "suicide" gene in the transformed cells. Most preferably, the cell carries the HSV-TK (herpes simplex virus thymidine kinase) gene as a safety measure, permitting the host cell to be killed *in vivo* by treatment with ganciclovir.

Use of a "suicide" gene is known in the art. See, e.g., Anderson, published PCT application WO 93/10218; Hamre, published PCT application WO 93/02556. The recipient's own immune system provides a first level of

protection from adverse reactions to the implanted cells. The presence of the TK gene (or other suicide gene) in the expression construct adds an additional level of safety to the recipient of the implanted cells.

In a further preferred embodiment, the Herpes Simplex Virus Thymidine kinase (HSV-TK) gene is inserted into the FasL vector construct. This suicide gene was included to allow elimination of the transfected cells upon ganciclovir administration. The 1800 bp fragment can be inserted into the NotI site of the pNUT vector. A typical dosage of ganciclovir required to "kill" the cells *in vivo* is approximately 5 mg/kg.

One preferred cell chosen for the gene transfer technique are Neuro-2A cells. While Neuro-2A cells are one preferred cell, a wide variety of cells may be used. It is required that the engineered cell does not express Fas/APO-1 receptor to ensure lack of "self-killing" and to ensure a high level of expression of FasL. For example, mouse Neuro-2A cells, do not express the Fas/APO-1 receptor.

Any other suitable cell type may also be used. The cells contemplated include well known, publicly available immortalized (and conditionally immortalized) cell lines as well as dividing primary cell cultures. Examples of suitable publicly available cell lines include, chinese hamster ovary (CHO), mouse fibroblast (L-M), NIH Swiss mouse embryo (NIH/3T3), African green monkey cell lines (including COS-1, COS-7, BSC-1, BSC-40, BMT-10 and Vero), rat adrenal pheochromocytoma (PC12 and PC12A), AT3, rat glial tumor (C6), astrocytes and other fibroblast cell lines, ß-TC cells, Hep-G2 cells, and myoblasts (including C_2C_{12} cells).

Primary cells that may be used include, neurospheres, neural progenitor cells and neural stem cells derived from the CNS of mammals (Richards et al., PNAS 89, pp. 8591-8595 (1992); Ray et al., PNAS 90, pp. 3602-3606 (1993)), primary fibroblasts, Schwann cells, astrocytes, and oligodendrocytes and their precursors. and the like. To the extent that any of the foregoing cells may

express the Fas/APO-1 receptor, the cells may be genetically modified to "knock out" expression of that receptor.

Cell lines offer several advantages including unlimited availability, the possibility of rapid screening *in vitro* for the presence of pathogens from which cell banks are established, and the suitability for stable gene transfer using non-viral-based recombinant DNA techniques.

Both allogeneic and xenogeneic cells may be used. Use of a cell line of xenogeneic origin provides an additional advantage since the transplanted cells are more likely to be rejected by the host immune system in the event of device breakage.

Typically the cells are surrounded with a membrane which permits the diffusion of small molecules such as nutrients and trophic factors into and out of the polymer envelope, while excluding larger molecules of the immune system (antibodies, complement, etc.).

Increased expression can be achieved by increasing or amplifying the copy number of the transgene encoding FasL or other suitable biologically active molecule(s), using amplification methods well known in the art. Such amplification methods include, e.g., DHFR amplification (see, e.g., Kaufman et al., United States patent 4,470,461) or glutamine synthetase ("GS") amplification (see, e.g., United States patent 5,122,464, and European published application EP 338,841).

In a preferred embodiment, the pNUT-FasL expression vector is transfected into Neuro-2A cells using a standard calcium phosphate transfection procedure and selected with increasing concentrations of methotrexate (1 to 200 μM) over 8 weeks to produce stable amplified cell lines. Following this selection, the engineered cells are maintained *in vitro* in 50-200 μM methotrexate.

According to this embodiment, FasL (or other apoptosis-inducing molecule) is continuously produced by the cells. While we prefer continuous production, we also contemplate regulated production of the desired apoptosis-inducing molecule.

We also contemplate co-delivery of other molecules.

For example, in the treatment of human tumors or cancers, co-delivery of FasL with one or more of lymphotoxin, TNF-α, and IFN-γ, is contemplated.

In the treatment of immune-mediated diseases, we contemplate co-delivery of FasL with neural growth factors, cytokines, hormones, and neurotransmitters (including peptide neurotransmitters). In particular, co-delivery of IGF-1, CNTF, GDNF, BDNF, NGF, NT-3, NT-4/5, NT-6, IFN-α, IFN-β, IL-4, IL-6, IL-10, CT-1, neuturin, persephin, TGF-β's, EGF and FGF is contemplated. It will be appreciated that any combination of the foregoing molecules may be co-delivered with FasL.

Co-delivery can be accomplished in a number of ways. Cells may be transfected with separate constructs containing the genes encoding the described molecules. Alternatively, cells may be transfected with a single construct containing two or more genes.

Multiple gene expression from a single transcript is preferred over that from multiple transcription units. One approach for achieving expression of multiple genes from a single eukaryotic transcript takes advantage of the 5' non-translated region in encephalomyocarditis virus (EMCV) known as the internal ribosome entry site ("IRES"). The IRES allows internal binding of ribosomes and translation of a downstream gene or genes. See, e.g., Macejak, Nature, 353, pp. 90-94 (1991); WO 94/24870; Mountford and Smith, Trends Genet., 11, pp. 179-84 (1995); Dirks et al., Gene, 128, pp. 247-49 (1993); Martinez-Salas et al., J. Virology, 67, pp. 3748-55 (1993); Mountford et al., Proc. Natl. Acad. Sci. USA, 91, pp. 4303-07 (1994); and Ghattas et al., Proc. Natl. Acad. Sci. USA, 91, pp. 5848-59 (1991). Any other suitable method known to one of ordinary skill in the art may also be used.

Also contemplated is encapsulation of two or more separately transfected cells or cell lines, each secreting one of the desired molecules.

This invention also contemplates use of different cell types during the course of the treatment regime. For example, a patient may be implanted with a

device device containing a first cell type (e.g., FasL-secreting Neuro-2A cells). If after time, the patient develops an immune response to that cell type, the device can be retrieved, or explanted, and a second device can be implanted containing a second cell type (e.g., FasL-secreting C_2C_{12} cells). In this manner, continuous provision of the therapeutic molecule is possible, even if the patient develops an immune response to one of the encapsulated cell types.

Encapsulation hinders elements of the immune system from entering the device, thereby protecting the encapsulated cells from immune destruction. This technology increases the diversity of cell types that can be employed in therapy. The semipermeable nature of the device membrane also permits the molecule of interest to easily diffuse from the device into the surrounding host tissue. This technique prevents the inherent risk of tumor formation and allows the use of unmatched human or even animal tissue, without immunosuppression of the recipient. Moreover, the implant may be retrieved if necessary or desired. It is both undesirable and expensive to maintain a patient in an immunosuppressed state for a substantial period of time. Such retrievability may be essential in many clinical situations.

Numerous encapsulation devices are known, having various outer surface morphologies and other mechanical and structural characteristics. Devices have been categorized as Type 1 (T1), Type 2 (T2), Type 1/2 (T1/2) or Type 4 (T4) depending on their outer surface morphology. Such membranes are described, e.g., in Lacy et al., "Maintenance Of Normoglycemia In Diabetic Mice By Subcutaneous Xenografts Of Encapsulated Islets", Science, 254, pp. 1782-84 (1991), Dionne et al., PCT/US92/03327 and Baetge et al., WO 95/05452.

As used herein "a biocompatible device" means that the device, upon implantation in a host mammal, does not elicit a detrimental host response sufficient to result in the rejection of the device or to render it inoperable, for example through degradation.

As used herein "an immunoisolatory device" means that the device upon implantation into a mammalian host minimizes the deleterious effects of the

host's immune system on the cells within its core or with the outer surface of the device.

A variety of biocompatible devices are suitable for delivery of molecules according to this invention. Such devices will allow for the passage of metabolites, nutrients and therapeutic substances while minimizing the detrimental effects of the host immune system. Preferably the device of this invention will be similar to those described in Aebischer et al., PCT publication WO 92/19195, incorporated herein by reference.

Useful biocompatible devices comprise (a) a core which contains a cell or cells, either suspended in a liquid medium or immobilized within a biocompatible matrix (e.g., a hydrogel or extracellular matrix), and (b) a surrounding or peripheral region of permeable matrix or membrane (jacket) which does not contain isolated cells, which is biocompatible. The jacket may be microporous or permselective.

The core of the polymer device is constructed to provide a suitable local environment for the continued viability and function of the cells isolated therein.

Many transformed cells or cell lines are most advantageously isolated within a device having a liquid core. For example, cells can be isolated within a device whose core comprises a nutrient medium, optionally containing a liquid source of additional factors to sustain cell viability and function. Further, the inner wall of the device may be coated with one or more molecules that facilitate cell adhesion. See, e.g., PCT/US95/09281.

Suitably, the core may be composed of a matrix formed by a hydrogel which stabilizes the position of the cells. The term "hydrogel" herein refers to a three dimensional network of cross-linked hydrophilic polymers. The network is in the form of a gel, substantially composed of water, preferably but not limited to gels being greater than 90% water.

Compositions which form hydrogels fall into three classes. The first class carries a net negative charge (e.g., alginate). The second class carries a net

positive charge (e.g., collagen and laminin). Examples of commercially available extracellular matrix components include Matrigel™ and Vitrogen™. Fibroblasts generally survive well in a positively charged matrix and are thus suitably enclosed in extracellular-matrix type hydrogels. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol). Any suitable matrix or spacer may be employed within the core, including agarose (including agarose derivatized with adhesion molecule fragments), precipitated chitosan, synthetic polymers and polymer blends, microcarriers and the like, depending upon the growth characteristics of the cells to be encapsulated.

Preferably, the devices are immunoisolatory. To be immunoisolatory, the surrounding or peripheral region of the device should confer protection of the cells from the immune system of the host in whom the device is implanted, by preventing harmful substances of the host's body from entering the core of the vehicle, and by providing a physical barrier sufficient to prevent detrimental immunological contact between the isolated cells and the host's immune system. The thickness of this physical barrier can vary, but it will always be sufficiently thick to prevent direct contact between the cells and/or substances on either side of the barrier. The thickness of this region generally ranges between 5 and 200 microns; thicknesses of 10 to 100 microns are preferred, and thickness of 20 to 75 microns are particularly preferred. Types of immunological attack which can be prevented or minimized by the use of the instant vehicle include attack by macrophages, neutrophils, cellular immune responses (e.g. natural killer cells and antibody-dependent T cell-mediated cytolysis (ADCC), and humoral response (e.g., antibody-dependent, complement-mediated cytolysis).

Use of immunoisolatory devices allows the implantation of xenogeneic cells or tissue, without a concomitant need to immunosuppress the recipient. The exclusion of IgG from the core of the vehicle is not the touchstone of immunoisolation, because in most cases IgG alone is insufficient to produce cytolysis of the target cells or tissues. Using immunoisolatory macrodevices, it is possible to deliver needed high molecular weight products or to provide metabolic

functions pertaining to high molecular weight substances, provided that critical substances necessary to the mediation of immunological attack are excluded from the immunoisolatory device. These substances may comprise the complement attack complex component Clq, or they may comprise phagocytic or cytotoxic cells; the instant immunoisolatory device provides a protective barrier between these harmful substances and the isolated cells. Nominal molecular weight cutoff (MWCO) values up to 1000 kD are contemplated. Preferably, the MWCO is between 50-700 kD. Most preferably, the MWCO is between 70-300 kD.

Various polymers and polymer blends can be used to manufacture the device jacket, including polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones (including polyether sulfones), polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

Alternatively the device jacket may be formed from any suitable biocompatible permeable material, including, e.g, hydrogels. See, e.g., WO92/19195.

The device can be any configuration appropriate for maintaining biological activity and providing access for delivery of the product or function, including for example, cylindrical, rectangular, disk-shaped, patch-shaped, ovoid, stellate, or spherical. Moreover, the device can be coiled or wrapped into a mesh-like or nested structure. If the device is to be retrieved after it is implanted, configurations which tend to lead to migration of the devices from the site of implantation, such as spherical devices small enough to migrate in the patient, are not preferred. Certain shapes, such as rectangles, patches, disks, cylinders, and flat sheets offer greater structural integrity and are preferable where retrieval is desired. For local delivery of FasL, e.g., in the treatment of solid tumors or cancers, a disk shape may be preferred.

Preferably the device has a tether that aids in retrieval. Such tethers are well known in the art. Such macrodevices have a core of a preferable minimum

volume of about 1 to 10µl and depending upon use are easily fabricated to have a volume in excess of 100 µl.

In a hollow fiber configuration, the fiber preferably has an inside diameter of less than 1500 microns, more preferably approximately 300-600 microns. If a semi-permeable membrane is used, the hydraulic permeability is preferably in the range of 1-100 mls/min/M^2/mmHg, more preferably in the range of 25 to 70 mls/min/M^2/mmHg. The glucose mass transfer coefficient of the device, defined, measured and calculated as described by Dionne et al., ASAIO Abstracts, p. 99 (1993), and Colton et al., The Kidney, eds., Brenner BM and Rector FC, pp. 2425-89 (1981) is preferably greater than 10^{-6} cm/sec, more preferably greater than 10^{-4} cm/sec.

Both semi-permeable membranes and microporous membranes are contemplated for forming the device jacket. Biocompatible, semi-permeable hollow fiber membranes, and methods of making them, are disclosed in United States patents 5,284,761, 5,158,881, and WO 95/0542, all herein incorporated by reference. In a preferred embodiment, the device is formed from a polyether sulfone membrane, such as those described in United States Patent Nos. 4,976,859 and 4,968,733 (describing both semi-permeable and microporous membranes), each herein incorporated by reference. See also Aebischer et al., J. Biomed. Engin., 113, p. 178 (1991).

Any suitable method of sealing the devices may be used, including the employment of polymer adhesives and/or crimping, knotting and heat sealing. These sealing techniques are known in the art. In addition, any suitable "dry" sealing method can also be used. In such methods, a substantially non-porous fitting is provided through which the cell-containing solution is introduced. Subsequent to filling, the device is sealed. Such a method is described in copending United States application Serial No. 08/082,407, herein incorporated by reference.

The methods and devices of this invention are intended for use in a mammalian host, recipient, patient, subject or individual, preferably a primate, most preferably a human.

In one embodiment, delivery of FasL is used as a potential treatment for glioblastoma. Glioblastomas are the most common form of malignant primary brain tumor. For people 15-34 years of age, it is the third leading cause of death from cancer. Little progress has been made in the treatment of glioblastoma. Conventional treatment consists of surgery to remove the bulk of the tumor followed by irradiation to ablate the remaining cancerous cells. Other approaches include chemotherapy and conventional gene therapy. Prognosis is poor. Median survival for patients with diagnosed glioblastoma is approximately nine to twelve months after diagnosis, with a five-year survival rate at only 5%.

For the treatment of brain tumors such as glioblastomas, we contemplate local delivery of FasL using an encapsulated source of FasL (such as encapsulated FasL-producing cells), or other apoptosis-inducing molecule, to the site of the tumor in the patient's CNS. Delivery to the brain ventricles, brain parenchyma or CSF is contemplated. Device placement is preferably proximate to or in the tumor tissue.

Other contemplated sites in the brain include the striatum, the cerebral cortex, subthalamic nuclei, nucleus Basalis of Maynert, and the cerebrospinal fluid ("CSF"), most preferably the subarachnoid space and the lateral ventricles.

The dosage of the apoptosis-inducing molecule can be varied by any suitable method known in the art. This includes changing the cellular production of FasL, achieved in any conventional manner, such as varying the copy number of the FasL gene in the transduced cell, or driving expression of the FasL using a higher or lower efficiency promoter, as desired. Further, the device volume and cell loading density can easily be varied, over at least three orders of magnitude. In addition, dosage may be controlled by implanting a fewer or greater number of devices. We prefer implanting between 1 and 10 devices per patient.

EXAMPLES

Example 1

This example shows that supernatants of murine FasL transfectants contain Fas/APO-1 specific cytotoxic activity which kills Fas/APO-1-expressing target cells *in vitro* and *in vivo*. Intraperitoneal application of doses which specifically killed encapsulated Yac-1 lymphoma cells implanted into the peritoneal cavity of mice was well tolerated and did not lead to liver damage.

In contrast, systemic administration of FasL-containing supernatant mimicked the effect of intraperitoneally applied anti-Fas/APO-1 antibody, causing liver failure due to hemorrhage and hepatocyte apoptosis.

Materials and Methods

Mice

A/J (H-2^a) mice were purchased from WIGA (Hannover, Germany). Balb/c (H-2^d) and C57BL/6 (H-2^b) mice were obtained from the breeding colony of the Institut für Zuchthygiene, Tierspital Zürich, Switzerland. All experiments were carried out with 6-10-wk-old mice of either sex.

Cell culture

The murine neuroblastoma cell line Neuro-2a and the murine lymphoma cell line Yac-1 were obtained from the American Type Culture Collection (Rockville, MD). Neuro-2a cells were maintained in MEM supplemented with 10% heat-inactivated FCS, 2mM L-glutamine, non-essential amino acids (lx) and penicillin (100 U/ml)/streptomycin (100 mg/ml) (GIBCO, Paisley, Scotland) and Yac-1 cells in RPMI 1640 (Hyclone, Cramlington, UK) containing 10% FCS, L-glutamine and antibiotics as above.

Reagents

Cycloheximide (CHX), acridine orange and protein A were obtained from Sigma Chemical Co. (St. Louis, MO). Hamster-anti-mouse Fas mAb Jo2 was from PharMingen (San Diego, CA), hamster IgG was purchased from Axell,

Accurate Chemical & Scientific Corporation (Westbury, NY). Soluble human Fas/APO-1 was kindly provided by Dr. P. Barr (Richmond, CA), $F(ab')_2$ fragments of anti-human APO-1 and control FH23 $F(ab')_2$ fragments were generated as described in Dhein et al., Nature, 373, p. 438 (1995).

FasL and Fas/APO-1 transfectants

The murine FasL cDNA was subcloned from pBS(-)KS (Stratagene, La Jolla, CA) into the *Xhol* and *NotI* sites of the BCMGS Neo expression plasmid (Karasuyama et al., J Exp. Med., 172, p. 969 (1990)). The human Fas/APO-1 cDNA was cloned into the same expression vector. Neuro-2a cells ($5x10^6$) were transfected with 10 µg plasmid DNA by electroporation using a Biorad Gene Pulser (25 kV). Selection with G418 (500 µg/ml) (GIBCO) was started 48 h later and continued throughout the culture period. Single clones were isolated 2-3 wks after transfection. Transcription of the FasL transgene was confirmed by RT-PCR. Expression of human Fas/APO-1 was determined by flow cytometry and Western blotting as previously described by Weller et al., J. Clin. Invest., 94, pp. 954 (1994). The cell-free supernatants were concentrated 100-fold using an Amicon stirred cell apparatus (Lexington, MA) with a YM10 membrane of 63 mm diameter (Amicon Corporation, Danvers, MA).

Reverse transcription-polymerase chain reaction (RT-PCR)

Reverse transcription and amplification were performed as previously described by Weller et al., Mol. Brain Res., 22, pp. 227 (1994). One primer was designed to bind to vector sequences and to the first 11 nucleotides of the FasL sequence corresponding to nucleotides 125-135 of the FasL sequence published by Takahashi et al., Cell, 76, pp. 969 (1994) (5'- TCC GCC ACC ATG CAG CAG CC -3') (SEQ ID NO. 1). The second primer bound to the vector sequences just 3'-prime to the FasL insert (5'- GTG GAT CCC CCG GGC TGC AG -3') (SEQ ID NO. 2). Thus the full-length FasL transgene but not the end genous FasL gene was amplified from reverse transcribed RNA of Neuro-2a FasL transfectants.

Cytotoxicity and proliferation assays

Yac-1 cells were seeded into 96-well microtiter F-plates (Falcon, Becton Dickinson, Oxnard, CA) at a density of 2×10^4 cells per well. The cells were stimulated with serial dilutions of 100-fold concentrated 24 to 48 h supernatant from Neuro-2a FasL and mock transfectants or with Jo2 or control hamster IgG. After 18 h, cultures were pulsed with 1 μCi (^{3}H)TdR (5 Ci/mmol; Amersham, Bucks. UK) per well for 6 h, harvested on filter paper strips using an automated sample harvester, and counted in a liquid β-scintillation counter. Survival of Neuro-2a human Fas/APO-1 and mock transfectants was determined by crystal violet staining after incubation for 16 h with the respective stimulant in the presence of cycloheximide (10 μg/ml).

SDS-PAGE and Western blot

SDS-PAGE of cell supernatants and Western blots were performed as described by Mariani et al., Eur. J. Immunol., 24, pp. 3119 (1994). Mouse Fas/APO-1 ligand specific rabbit antiserum was produced according to routine procedures. Horseradish peroxidase (HRPO)-conjugated mouse anti-rabbit IgG (H+L) Ab (Dianova, Hamburg, Germany) was used as a detecting reagent.

Surgical procedure

To study the effects of the FasL on tumor cells *in vivo*, Yac-1 cells enclosed in permeable devices were implanted into the peritoneum of mice. Semi-permeable polyether sulfone hollow fiber membranes (outside diameter: 600 μm) with a molecular weight cut-off of 2×10^3 kD were loaded with 10^5 cells/μl. See, e.g., Aebischer et al., J. Biomed. Engin., 113, p. 178 (1991). Each membrane device contained 4-6 x 10^5 Yac-1 cells. The mice were anesthetized by intramuscular injection of Innovar-Vet (0.16 mg sublimazine/kg and 8 mg inapsine/kg) and were placed in a sterile working unit for the surgical procedure. The peritoneum was opened by a small incision, two to maximally four devices were placed in the peritoneal cavity and the skin closed with two wound clips. Eight hours later the mice were injected i.p. with concentrated FasL or control supernatant in a volume of 2 ml.

Viability staining and detection of apoptosis

After exposure to FasL supernatant *in vitro* or *in vivo*, Yac-1 cells were removed from the devices and incubated with fluorescein diacetate (FDA) at a final concentration of 10 µg/ml for 5 min. Subsequently, viable and dead cells were counted by fluorescence microscopy and percentage viability was calculated. The morphology of dead cells was monitored by acridine orange nuclear staining. DNA fragmentation was examined by *in situ* DNA end labeling (Gavrieli et al., J. Cell. Biol., 119, p. 493 (1992)) using terminal transferase-mediated incorporation of biotinylated deoxyuridine triphosphate (50 µM) and streptavidin-alkaline phosphatase detection (all Boehringer Mannheim, Germany).

Results and Discussion

For the production of recombinant FasL, Neuro-2a cells were transfected with the CDNA encoding murine FasL. Neuro-2a cells were chosen because they do not express Fas/APO-1 as determined by flow cytometry and are resistant to Fas/APO-1 mediated cytotoxicity (data shown below). Successful transfection was confirmed by reverse transcription polymerase chain reaction (RT-PCR). A band of the expected size was seen in FasL transfectants but not in Neuro-2a cells transfected with the empty vector (Fig. 1a).

To determine whether active FasL was present in the supernatant, culture medium of subconfluent FasL and mock transfectants was collected, concentrated 100-fold and administered to Yac-1 lymphoma cells, which express Fas/APO-1 and are known to be sensitive to Fas/APO-1-mediated cytotoxicity.

Yac-1 cells were incubated with serial dilutions of supernatant for 24 h and proliferation assessed by (^{3}H) thymidine incorporation during the last 6 h of the incubation period. Supernatant of FasL transfectants, but not of mock transfectants, was highly cytotoxic for Yac-1 cells (Fig. 1b). The FasL concentration which induced 50% cell killing of Yac-1 cells was defined as 1 unit/ml.

Yac-1 cells were also exposed to the antiFas/APO-1 antibody Jo2 (see, e.g., Ogasawara et al., Nature, 364, p. 806 (1994)), which was clearly less

effective: concentrations of up to 100 µg/ml caused only approximately 20% cell death or 30% cell death in the presence of protein A (Fig. 1c).

Protein A promotes Fc-Fc interactions of antibodies and increases their ability to cross-link Fas/APO-1 receptors on the target cell surface. Receptor multimerization appears to be required for the generation of the apoptotic signal. Adsorption of anti-Fas/APO-1 antibody to a solid phase support has also been described to enhance its cytotoxic activity but failed to augment killing of Yac-1 cells in our experiments (data not shown).

To rule out effects of unspecific cytotoxic factors, the supernatant was tested on Fas/APO-1 positive and negative target cells. For this purpose, Neuro-2a cells were transfected with the human Fas/APO-1 cDNA or with the vector control. Mouse FasL has been shown to bind to human Fas/APO-1. Stable transfectants should differ only in their expression level of human Fas/APO-1 and as a consequence in their susceptibility to Fas/APO-1-mediated apoptosis. Indeed, supernatant of FasL transfectants efficiently killed Neuro-2a target cells expressing human Fas/APO-1 (Fig. 1d), but did not affect viability of the mock transfectants (data not shown).

The specificity of the cytotoxic effect of the supernatant was further determined by adding soluble human Fas/APO-1 (sFas) or anti-Fas/APO-1 antibody $F(ab')_2$ fragments (see Dhein et al., Nature, 373, p. 438 (1995)) to the FasL cytotoxicity assay. Both reagents would be expected to specifically block FasL-mediated cell death either by scavenging FasL from the supernatant or by competing with the binding of FasL to Fas/APO-1 on the target cells.

As demonstrated in Fig. 1b, sFas completely blocked supernatant-mediated cytotoxicity on Yac-1 cells at a concentration of 500 ng/ml, while soluble TNF-receptor (sTNF-R) had no inhibitory effect.

Similarly, $F(ab')_2$ anti-Fas/APO-1 reduced supernatant-induced cell killing of Neuro-2a Fas/APO-1 transfectants at a concentration of 10 µg/ml (Fig. 1d). Inhibition by both reagents was dose-dependent (data not shown) and specific

since no effect was seen after the addition of sTNF-R or FII23 control $F(ab')_2$ fragments (Fig. 1d).

To further confirm that the cytotoxic activity of the supernatant can be attributed to FasL, Western blot analysis was carried out using antibodies directed to murine FasL (Fig. 2). Only in the supernatant derived from Neuro-2a FasL (lane 1), but not in that from Neuro-2a mock transfectants (lane 2), a specific band of 40 kD was detected. The size of the specific band suggests that the supernatant contains the full-length FasL and not a proteolytically cleaved form of the molecule. The minor band of 20 kD may reflect FasL degradation products.

To determine the cytotoxic activity of the FasL *in vivo*, we implanted devices containing defined numbers of Yac-1 cells into the peritoneal cavity of syngeneic A/J mice ($H\text{-}2^a$). These devices are permeable for macromolecules of up to 2×10^3 kD but not for cells, thus enabling us to precisely trace the fate of the implanted tumor cells. Two devices each containing 4 to 6×10^5 Yac-1 cells were implanted into each mouse. Eight hours after implantation, mice were injected i.p. with increasing amounts of FasL-containing supernatant or control supernatant. Sixteen hours later, mice were sacrificed and the Yac-1 cell-containing devices removed for further analysis. None of the mice had complications from the surgical procedure or signs of irritation from the devices, reconfirming the biocompatibility of the membrane material. See, e.g., Christenson et al., Biomed. Mater. Res., 25, p. 1119 (1991).

In parallel, encapsulated Yac-1 cells were exposed to FasL-containing supernatant or control supernatant for 16 h *in vitro*. Viability of the Yac-1 cells was assessed by FDA staining. As shown in Fig. 3a, FasL-containing supernatant reduced viability in a dose-dependent manner *in vivo*. The maximal dose of 2000 U resulted in 100% cell killing, while equally concentrated supernatant of mock transfectants did not impair viability. Thus, the FasL supernatant is also cytotoxic *in vivo*. Efficiency of FasL cell killing was dependent on the FasL concentration but not on the number of target cells, at least in the tested range of 5×10^5 to 2×10^6 target cells (data not shown). Implantation of

higher target cell numbers i.p. was limited by the size of the devices and the peritoneal space. As expected, FasL supernatant also efficiently killed encapsulated Yac-1 cells *in vitro* (data not shown). The experiments demonstrate that the FasL is principally cytotoxic *in vivo*, however, the efficiency of tumor cell killing in naturally growing solid tumors using FasL-secreting encapsulated cells has to be further evaluated.

Although the anti-Fas/APO-1 antibody Jo2 did not efficiently kill Yac-1 cells *in vitro*, it was possible that it would be much more active *in vivo*. We thus injected sublethal doses of antibody (1-5 μg/mouse) i.p. and subsequently analyzed viability of the implanted Yac-1 cells. In accordance with the *in vitro* results these antibody doses were not cytotoxic for Yac-1 targets (data not shown).

To confirm that FasL-induced cell death was apoptotic, we analyzed cell morphology and DNA integrity in Yac-1 cells isolated from the devices after *in vivo* treatment. Fig. 3b demonstrates that FasL caused nuclear condensation and fragmentation of Yac-1 cells, while the nuclei of cells treated with control supernatant were intact (Fig. 3c). Furthermore, *in situ* DNA end labeling revealed that DNA strand breaks, another hallmark of apoptosis, were dramatically increased only in FasL-treated cells (Fig. 3d and e).

It has previously been shown that a single i.p. injection of 100 μg of anti-Fas/APO-1 antibodies induces lethal liver cell apoptosis within 6 hours after injection. In contrast, we show here that mice injected i.p. with FasL-containing supernatant clinically appeared completely normal. In order to detect possible minor histological changes, tissue sections were examined at various time points from 4 to 24 h after FasL application. Up to 2000 units of FasL injected into A/J mice did not cause histological changes in the liver (Fig. 4a).

FasL supernatant was given also to Balb/c and C57BL/6 mice. Likewise, i.p. application of FasL was well tolerated in these mice and liver sections were normal.

The difference of FasL compared to anti-Fas/APO-1 antibodies is especially striking since a dose of FasL which efficiently kills 100% of the Yac-1

cells *in vivo* has no systemic side effects when applied i.p., while a dose of anti-Fas/APO-1 antibodies which only kills about 20% of Yac-1 cells *in vitro* induces lethal liver toxicity *in vivo*.

FasL caused severe liver hemorrhage and hepatocyte apoptosis when injected i.v. In fact, if applied by this route, it induced changes analogous to those seen after i.p. administration of anti-Fas/APO-1 antibody (Fig. 4b and d). Histology revealed altered liver tissue with dilated sinusoids, loss of sinusoidal endothelial cells and hemorrhages. Damaged hepatocytes of irregular size exhibited condensed and fragmented nuclei indicative of apoptosis.

In contrast, i.v. injection of concentrated control supernatant did not induce liver damage (Fig. 4c). Normal liver tissue with regular trabecules and slight steatosis was observed. Thus, FasL given i.p. acts locally on target cells without inducing hepatocyte apoptosis.

Example 2

In the above experiment, Yac-1 cells (which bear the Fas/APO-1 receptor) were encapsulated, and the killing effect of injected soluble FasL on those cells was demonstrated. In further experiments, the FasL-secreting cells are encapsulated and the effect of the secreted FasL on tumor tissue is demonstrated, both *in vitro* and *in vivo*. For these experiments, the following encapsulation procedure is suitable.

Encapsulation

Hollow fibers are fabricated from a polyether sulfone (PES) with an outside diameter of 720 µm and a wall thickness of a 100 µm (AKZO-Nobel Wüppertal, Germany). These fibers are described in United States patents 4,976,859 and 4,968,733, herein incorporated by reference. In some studies, including human studies, we contemplate using a polyether sulfone ("PES") PES#5 membrane which has a MWCO of about 280 kd. In other studies we contemplate using a PES#8 membrane which has a MWCO of about 90 kd.

The devices comprise:

1) a semipermeable polyether sulfone hollow fiber membrane fabricated by AKZO Nobel Faser AG;

2) a hub membrane segment;

3) a light cured methacrylate (LCM) resin leading end; and

4) a silicone tether.

The semipermeable PES membrane contemplated in the human studies has the following characteristics:

Internal Diameter	500±30 μm
Wall Thickness	100 ±15 μm
Force at Break	100±15 cN
Elongation at Break	44±10%
Hydraulic Permeability (ml/min m^2 mmHg)	63±8
nMWCO (dextrans)	280±20 kd

The components of the device are commercially available. The LCM glue is available from Ablestik Laboratories (Newark, DE); Luxtrak Adhesives LCM23 and LCM24). The tether material is available from Specialty Silicone Fabricators (Robles, CA). The tether dimensions are 0.79 mm OD x 0.43 mm ID x length 202 mm.

Fiber material is cut into 5 cm long segment and the distal end of the devices are sealed with a photopolymerized acrylic glue (LCM-25, ICI). Following sterilization with ethylene oxide and outgassing, the fiber segments are loaded with a suspension of between 10^3 to 10^7 cells via a Hamilton syringe and a 25 gauge needle through an attached injection port. In some studies we use a collagen matrix, e.g., Zyplast™.

The proximal end of the device is sealed with the same acrylic glue. The volume of the device is approximately 15-18 μl.

A silicone tether (Specialty Silicone Fabrication, Taunton, MA) (ID: 690 μm; OD: 1.25 μm) is placed over the proximal end of the fiber allowing easy manipulation and retrieval of the device.

Human Clinical Trial

A membrane-based implant, containing between 10^3 to 10^7 cells producing FasL, as described above, is implanted into the tumor bed in the CNS and/or into the CSF fluid compartment.

Protocol

Patient recruitment may be based on the following criteria:

a) Entry criteria:

(1) A diagnosis of high grade glioma (WHO III and IV), manifested clinically by elevated intracranial pressure and focal signs such as epilepsy or paresis of limbs and cranial nerves and confirmed by CT and/or MRI findings.

b) Exclusion criteria

(1) Patient with a life threatening illness (eg, cancer, leukemia) in addition to glioblastoma; (2) Pregnant woman or woman of child-bearing potential without adequate contraception; (3) Patient with neurological involvement outside the voluntary motor system; (4) Evidence of primary disease that could cause a neurologic deficit (in particular, cervical spondylosis or plasma cell dyscrasia); (5) Patient participating in any investigation drug trial running concurrently to this trial; (6) patients with clinical or chemical evidence for liver disease.

Implantation:

Patients receive a hollow fiber implant or a round implant (e.g., shaped as a disk) containing FasL-producing cells. Standard surgical procedure, such as that described in WO 94/15663, may be used to implant the devices of this invention.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: METHOD AND DEVICE FOR DELIVERY OF APOPTOSIS-INDUCING MOLECULES

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/633,709
(B) FILING DATE: 17-APR-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TCCGCCACCA TGCAGCAGCC 20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTGGATCCCC CGGGCTGCAG 20

WE CLAIM:

1. A method for delivering an apoptosis-inducing molecule to a patient comprising implanting into said patient a device containing a cellular source of the apoptosis-inducing molecule.

2. The method of claim 1 wherein the device is implanted directly into the central nervous system of the patient.

3. The method of claim 1 wherein the device is implanted into the brain parenchyma.

4. The method of claim 1 wherein the device is implanted into the cerebrospinal fluid.

5. The method of claim 1 wherein the apoptosis-inducing molecule is selected from the group consisting of FasL, lymphotoxin, TNF-α, and IFN-γ.

6. The method of claim 1 wherein the apoptosis-inducing molecule is FasL.

7. A device that produces an apoptosis-inducing molecule, the device comprising:

(a) a core comprising cells that produce an apoptosis-inducing molecule; and

(b) a jacket surrounding said core, the jacket comprising a permeable membrane that permits passage of the apoptosis-inducing molecule thereacross.

8. The device of claim 7 wherein the apoptosis-inducing molecule is selected from the group consisting of FasL, lymphotoxin, TNF-α, and IFN-γ.

9. The device of claim 8 wherein the apoptosis inducing molecule is FasL.

10. The device of claim 7 wherein the cells in the core are dispersed in a biocompatible matrix.

11. The device of claim 7 wherein the core additionally comprises a cellular source of a second biologically active molecule.

12. A method for treating a human tumor or cancer comprising implanting one or more devices into a patient, at least one device containing cells that produce an apoptosis-inducing molecule, said molecule inducing, at least in part, apoptosis of the tumor or cancer, but not of the surrounding non-tumorigenic or non-cancerous tissue in the patient.

13. The method of claim 12 wherein the apoptosis-inducing molecule is selected from the group consisting of FasL, lymphotoxin, TNF-α, and IFN-γ.

14. The method of claim 13 wherein the apoptosis-inducing molecule is FasL.

15. The method of claim 12 wherein the human tumor or cancer is located in the central nervous system, and the device is implanted into the central nervous system.

16. The method of claim 12 wherein at least one device contains cells that produce a second biologically active molecule.

17. The method of claim 16 wherein the second biologically active molecule is selected from the group consisting of lymphotoxin, TNF-α, and IFN-γ.

18. A method for treating an autoimmune disease in the central nervous system comprising implanting one or more devices into the central nervous system of a patient, at least one device containing a cellular source of an apoptosis-inducing molecule in sufficient dosage to result in destruction, at least partially, of activated cytotoxic lymphocytes.

19. The method of claim 18 wherein the apoptosis-inducing molecule is selected from the group consisting of FasL, lymphotoxin, TNF-α, and IFN-γ.

20. The method of claim 19 wherein the apoptosis-inducing molecule is FasL.

21. The method of claim 18 wherein at least one device contains a cellular source of a second biologically active molecule.

22. The method of claim 21 wherein the second biologically active molecule is selected from the group consisting of neural growth factors, cytokines, hormones, neurotransmitters and peptide neurotransmitters.

23. The method of claim 21 wherein the second biologically active molecule is selected from the group consisting of IGF-1, CNTF, GDNF,

BDNF, NGF, NT-3, NT-4/5, NT-6, IFN-α, IFN-β, IL-4, IL-6, IL-10, CT-1, neuturin, persephin, TGF-β's, EGF and FGF.

24. A method for treating a human tumor or cancer as an adjunct to treatment with a chemotherapeutic agent, the method comprising implanting one or more devices into a patient, at least one device containing cells that produce an apoptosis-inducing molecule, said molecule inducing, at least in part, apoptosis of the tumor or cancer, but not of the surrounding non-tumorigenic or non-cancerous tissue in the patient.

25. A composition for treating a human tumor or cancer as an adjunct to treatment with a chemotherapeutic agent, the composition comprising one or more devices, at least one device containing cells that produce an apoptosis-inducing molecule, said molecule inducing, at least in part, apoptosis of the tumor or cancer, but not of the surrounding non-tumorigenic or non-cancerous tissue when implanted into the patient.

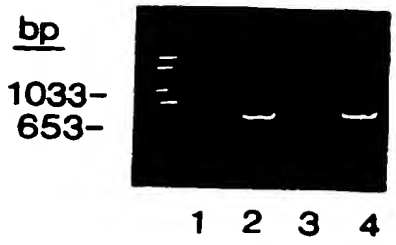

FIG. 1A

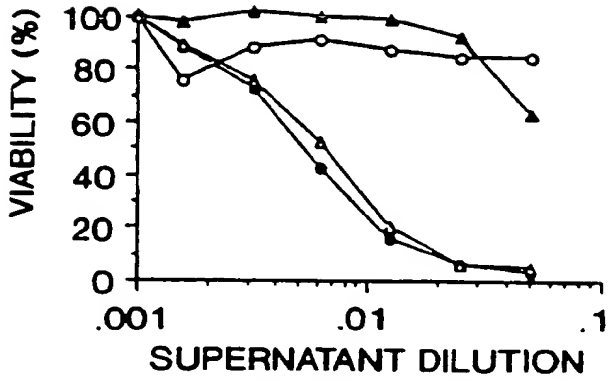

FIG. 1B

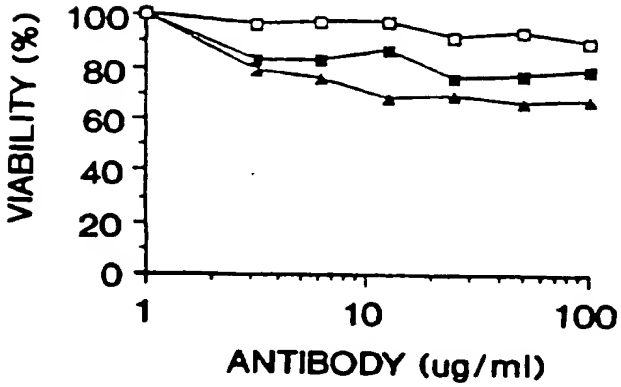

FIG. 1C

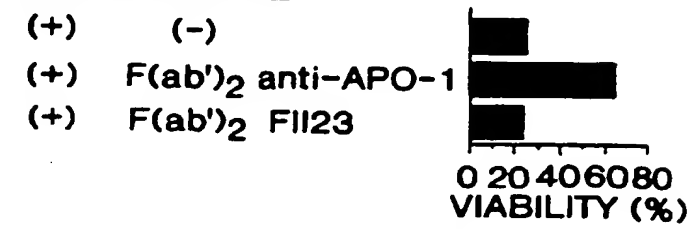

FIG. 1D

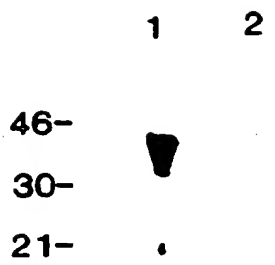

FIG. 2

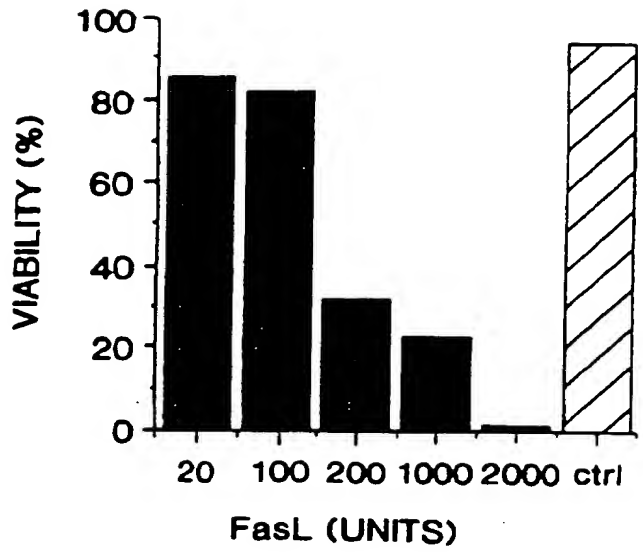
100
80
60
40
20
0
VIABILITY (%)
20
100
200
1000
2000
ctrl
FasL (UNITS)

FIG. 3A

FIG. 3C

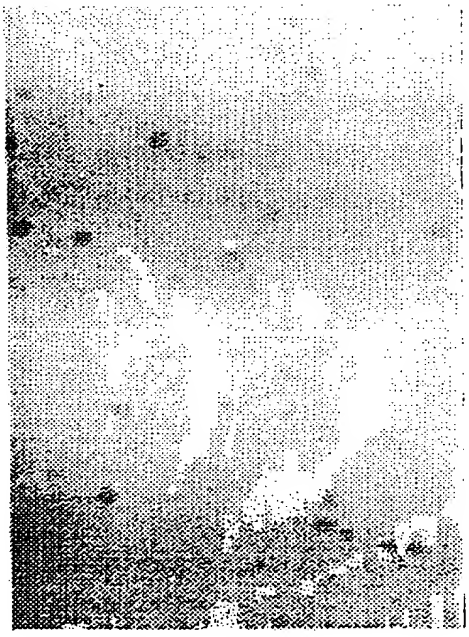

FIG. 3E

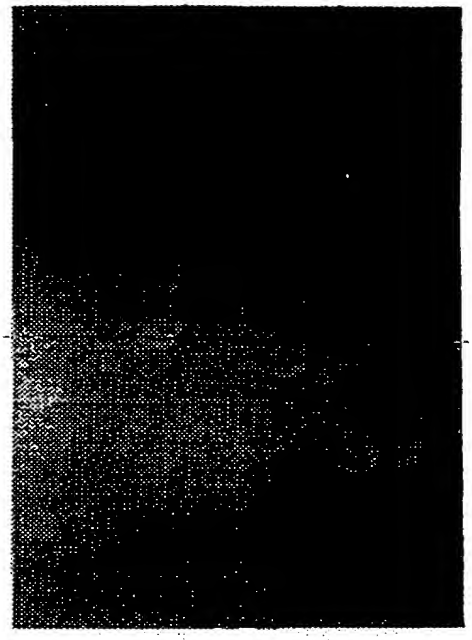

FIG. 3B

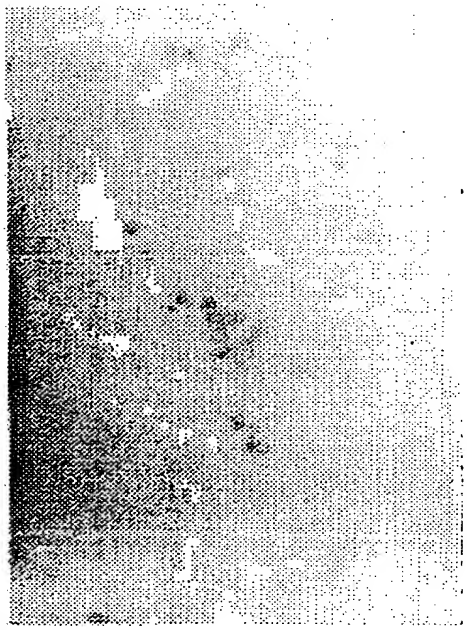

FIG. 3D

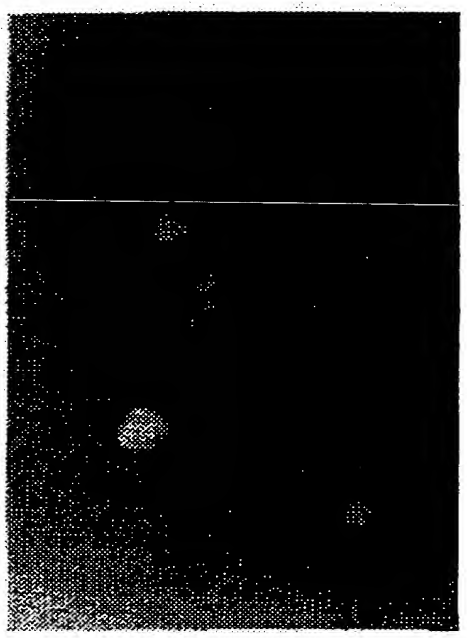

FIG. 4B

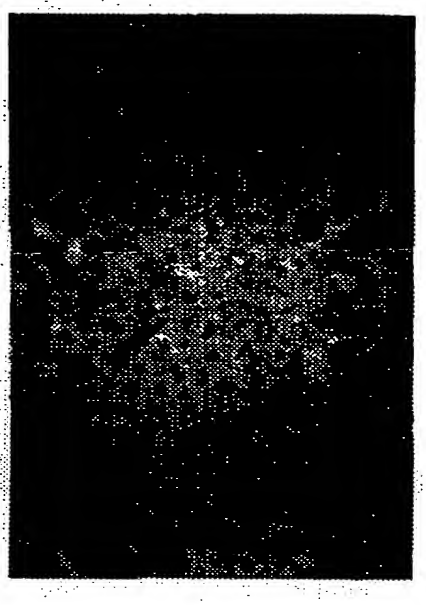

FIG. 4D

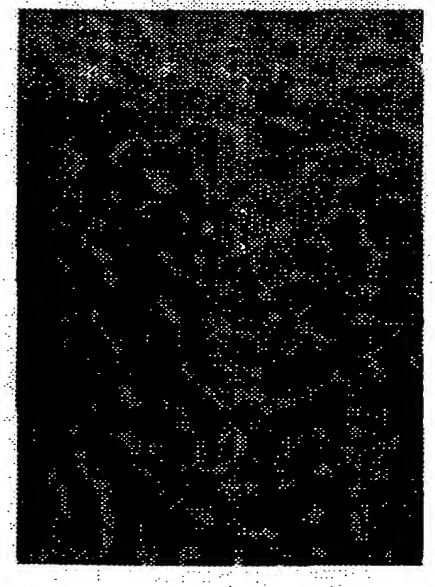

FIG. 4A

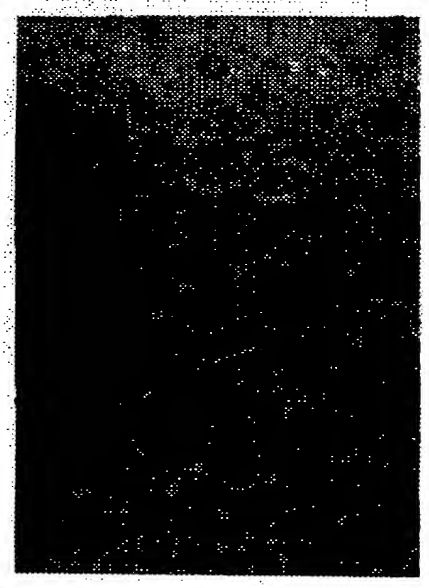

FIG. 4C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/06409

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 A61K38/19 A61K38/21 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF EXPERIMENTAL MEDICINE, vol. 181, no. 1, 1 January 1995, pages 71-77, XP000645126 ALDERSON M R ET AL: "FAS LIGAND MEDIATES ACTIVATION-INDUCED CELL DEATH IN HUMAN T LYMPHOCYTES" see the whole document ---	1-25
X	SCIENCE, vol. 267, 10 March 1995, pages 1449-1456, XP002026333 NAGATA S ET AL: "THE FAS DEATH FACTOR" see the whole document ---	1-25
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 July 1997	07. 08. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Sitch, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/06409

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 05452 A (CYTOTHERAPEUTICS INC ;BAETGE E EDWARD (US); HAMMANG JOSEPH P (US);) 23 February 1995 cited in the application see page 12, line 2 - page 39, line 34 ---	
A	WO 92 19195 A (UNIV BROWN RES FOUND) 12 November 1992 cited in the application see page 4, line 5 - page 11, line 24 ---	
A	WO 95 16464 A (UNIV JOHNS HOPKINS MED) 22 June 1995 see page 7, line 2 - line 27 ---	
A	CANCER RESEARCH, vol. 55, 1 July 1995, pages 2936-2944, XP002036423 WELLER ET AL: "FAS/APO-1 GENE TRANSFER FOR HUMAN MALIGNANT GLIOMA" see the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/06409

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-6,12-24
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-6,12-24 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/06409

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9505452 A	23-02-95	AU 7568094 A	14-03-95
		CA 2169292 A	23-02-95
		FI 960611 A	09-04-96
		NO 960547 A	12-04-96
		US 5639275 A	17-06-97
WO 9219195 A	12-11-92	AU 666118 B	01-02-96
		AU 2004192 A	21-12-92
		AU 3902095 A	01-02-96
		EP 0585368 A	09-03-94
		JP 6507412 T	25-08-94
		NO 933842 A	25-10-93
WO 9516464 A	22-06-95	CA 2178902 A	22-06-95
		EP 0741580 A	13-11-96